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# Selectin ligands, leukocyte trafficking, and fucosyltransferase genes

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Circulating leukocytes leave the intravascular compartment via events that include the participation of sequentially-acting leukocyte-endothelial adhesion receptor-counter-receptor pairs [reviewed in 1]. Initial events in this process are characterized by oligosaccharide-dependent adhesive interactions. In the context of vascular shear flow, these interactions promote the rolling of leukocytes along the endothelial surface of the blood vessel interior. Leukocyte rolling is in turn a prerequisite for subsequent, activation-dependent events leading to integrin-mediated leukocyte arrest, and endothelial transmigration.

Leukocyte rolling is a property characteristic of adhesion mediated by E-, P-, and L-selectins. As will be detailed below, these glycoproteins express structurally similar NH2-terminal carbohydrate binding domains, are encoded by closely linked loci, and maintain similar, though distinct counter-receptor requirements [1, 2]. Given the important role assigned to selectin-dependent leukocyte adhesion in inflammation, and attendant opportunities for therapeutic intervention in inflammation-dependent pathology, there has been a substantial amount of interest in defining the molecular nature of the ligands for E-, P-, and L-selectins. This manuscript will outline the current understanding of the molecules that function as selectin ligands, and on how the construction of these molecules is accomplished, and regulated, especially via the actions of a specific $\alpha$(1,3)fucosyltransferase termed Fuc-TVII.

## L-selectin ligands

Mammalian lymphocytes recirculate between the intravascular compartment and the lymphatic system. Lymphocytes leave the vascular tree within lymph nodes, and other lymphoid aggregates like Peyer's patches [reviewed in 1, 3, 4]. Lymphocytes that have emerged into these secondary lymphoid organs pass through the parenchyma of the lymphoid aggregate, travel in the lymphatic ductal system, and return to the vascular tree at the thoracic duct. The lymphocyte recirculatory system facilitates lymphocyte encounters with foreign antigens within the lymphatic organs, and comprises an essential part of the normal immune system. Lymphocytes depart the circulation through adhesion-dependent processes occurring in the postcapillary venules of secondary lymphoid organs. The initial events in this process, especially with respect to lymphocyte homing to peripheral lymph nodes, are characterized by adhesion between L-selectin expressed by the lymphocytes, and L-selectin counter receptors expressed by specialized endothelial cells lining the postcapillary venules [reviewed in 1, 3]. These specialized postcapillary venular endothelial cells are cuboidal in shape, and are functionally distinct from typical vascular endothelia. The venules lined with these cells are termed high endothelial venules, or HEVs. Lymphocyte homing to the peripheral lymph nodes is mediated in large measure by oligosaccharide-dependent adhesive processes that occur between circulating lymphocytes and the peripheral node HEV (PNHEV).

Functional assays reflective of lymphocyte homing include the Stamper-Woodruff assay [5]. This assay involves application of lymphocytes to HEVs exposed at the surface of a frozen section of a peripheral lymph node. Adherent cells remaining attached to the HEV after washing are quantitated, as a reflection of lymphocyte adhesion to the exposed HEV. This assay was used together with biochemical and immunologic approaches to identity a lymphocyte cell surface antigen, recognized by the monoclonal antibody MEL-14, that mediates lymphocyte adhesion to PNHEV [6, 7]. The MEL-14 monoclonal antibody blocks lymphocyte adhesion to PNHEV and inhibits homing of lymphocytes to the peripheral lymph nodes *in vivo* [6].

Early studies provided circumstantial evidence for the possibility that MEL-14 mediates lymphocyte adhesion through recognition of carbohydrate determinants displayed by PNHEV [8, 9]. This hypothesis was prompted in part by the observation that the surface of PNHEV is composed in part of a prominent glycocalyx, and by the fact that glutaraldehyde fixation of the exposed PNHEV, which generally leaves carbohydrate determinants intact, while denaturing many polypeptide antigens, minimally diminishes lymphocyte adhesion to PNHEV [5]. The notion that MEL-14-dependent lymphocyte adhesion is dependent upon specific carbohydrate determinants was supported by a series of experimental observations [8, 9] indicating that adhesion could be blocked by some monosaccharides, and by experimental manipulations involving removal of sialic acid moieties from the surface of PNHEV with sialidases [9–11]. This work and other studies [reviewed in 3] indirectly implicating the MEL-14 antigen as a carbohydrate binding protein, implied that lymphocyte-PNHEV adhesive interactions were mediated by the molecular interactions between the adhesion molecule on lymphocytes recognized by the MEL-14 monoclonal antibody, and sialylated carbohydrate-containing counter-receptors on PNHEV.

Molecular cloning studies subsequently demonstrated that the MEL-14 antigen corresponds to a protein now termed L-selectin (cluster designation CD62L) [12–17]. The polypeptide sequence

of the amino terminal domain of L-selectin is similar to a family of proteins, termed C-type lectins, that exhibit calcium-dependent carbohydrate binding properties [18]. This information provided additional strong evidence that the calcium-dependent nature of lymphocyte-PNHEV adhesive interactions was mediated through L-selectin recognition of specific PNHEV-borne, carbohydrate-containing counter-receptors.

More recent work illuminates the nature of a series of glycoproteins expressed by PNHEV that function as L-selectin counter-receptors, and has identified candidate oligosaccharide structures required for L-selectin-dependent recognition of these molecules. Some of this work has employed a recombinant L-selectin-IgG chimera as an immunohistochemical probe to explore the nature of these ligands in tissues sections, and as an affinity purification reagent for biochemical characterization [19, 20]. This work has led to the molecular cloning of cDNAs encoding several PNHEV glycoproteins with L-selectin counter-receptor activity. In immunochemical studies, the L-selectin-Ig chimera immunoprecipitates 50 kDa and 90 kDa [$^{35}$S]-labeled polypeptides in extracts prepared from lymph nodes radiolabeled with [$^{35}$S]sulfate [20–22]. Both immunoprecipitated molecules can be radiolabeled with fucose, but not with mannose or glucose, indicating that they are sulfated, fucosylated glycoproteins. The inability to label these molecules with mannose or glucose, together with the fact that their electrophoretic mobilities were not altered by digestion with N-glycanase, implied that the glycoproteins contain few, if any, asparagine-linked (N-linked) oligosaccharides [23]. These data suggest that most, and perhaps all, of the glycans displayed by these two proteins are of the serine/threonine-linked type (O-linked glycans). These molecules were initially termed Sgp$^{50}$ and Sgp$^{90}$, for sulfated glycoprotein of 50 kDa and 90 kDa, respectively. The L-selectin-IgG chimera immunoprecipitates Sgp$^{50}$ and Sgp$^{90}$ even after their extraction from denaturing SDS polyacrylamide gels, implying that their recognition by the L-selectin-IgG chimera occurs primarily through the glycan moieties. Similar approaches identify a third molecule, termed Sgp$^{200}$, with similar biochemical properties, in lymph node extracts [24].

Sgp$^{50}$ and Sgp$^{90}$ have been purified, and cDNAs encoding these molecules have been cloned [25, 26]. The Sgp$^{50}$ cDNA predicts a 132 amino acid long polypeptide, known as GlyCAM-1, for glycosylation-dependent cell adhesion molecule. The sequence predicted for GlyCAM-1 includes numerous serine and threonine residues with potential for modification by O-glycosylation, but only a single potential N-linked glycosylation site [25]. The numerous serine and threonine residues, when considered together with the difference in size between the observed (~50,000 Da) and predicted (14,154 Da) molecular masses, suggests that O-linked glycans make up a large portion of the glycoprotein's size, and recommend it as a mucin-type glycoprotein. Immunoelectron microscopy studies show that little, if any, GlyCAM-1 is expressed at the surface of the HEV. However, it is certainly released as a soluble molecule following passage through the secretory pathway [27], confirming the observation that GlyCAM-1 is detectable as a soluble entity in the conditioned medium of lymph node organ cultures [25]. Absence of membrane association is consistent with the absence of a clearly evident membrane spanning domain within the peptide sequence of GlyCAM-1. These observations suggest that GlyCAM-1 does not directly mediate L-selectin-dependent adhesion of lymphocytes to HEV. The function of this soluble counter-receptor for L-selectin remains to be defined.

Protein sequence analysis of Sgp$^{90}$ affinity purified from mouse peripheral lymph nodes disclosed peptide sequences corresponding to the previously cloned murine sialomucin molecule CD34 [26]. The predicted structure of CD34 is similar to that of GlyCAM-1 in that it contains numerous serine and threonine-rich, mucin-like domains that are apparently heavily modified by O-linked glycan structures. Biochemical analyses referred to above [21, 24] indicates that the O-glycans on PLN-derived CD34/Sgp$^{90}$ are sulfated, and, as with GlyCAM-1, are probably essential to recognition by L-selectin. Since CD34 maintains a single membrane-spanning domain that places the bulk of the molecule on the extracellular face of the plasma membrane, it seems probable that it figures importantly in the L-selectin-dependent adhesion of lymphocytes to lymph node HEV.

Since CD34 is expressed by vascular endothelial cells, and by many other tissues [28], it is possible that CD34 could operate as an L-selectin counter-receptor to facilitate leukocyte-endothelial cell interactions in acute and chronic inflammation (provided it has been glycosylated appropriately). This notion has received some support recently, through the observation that L-selectin-dependent leukocyte rolling *in vitro* can be supported by some glycoforms of CD34 in the human tonsil [29].

A third molecule, known as the mucosal vascular addressin molecule MAdCAM-1, can also present oligosaccharide ligands to L-selectin [30]. MAdCAM-1 functions as the counter-receptor for the lymphocyte homing receptor $\alpha4\beta7$, and directs a large fraction of the lymphocyte homing to Peyer's patches [31]. MAdCAM-1 isolated from mouse mesenteric lymph nodes displays sialylated, N-glycanase-resistant glycans, and can support L-selectin-dependent leukocyte rolling *in vitro*. By contrast, glycoforms of MAdCAM-1 isolated from cells where L-selectin-dependent adhesion is not observed do not support L-selectin-dependent leukocyte rolling. This work implies that tissue-specific glycosylation of MAdCAM-1 determines its function as an L-selectin counter-receptor. These observations additionally imply that MAdCAM-1 can operate both as a counter-receptor for shear-dependent adhesion processes (mediated by both L-selectin and the $\alpha4\beta7$ integrin), as well as a counter-receptor for subsequent firm attachment events (also mediated by $\alpha4\beta7$ integrin).

The nature of the glycan moieties that clearly play an important role in L-selectin-dependent interactions has also received a considerable amount of recent attention. As noted above, early studies implied a critical role for sialic acid moieties in these interactions. Studies with sialidases with specificity for different sialic acid linkages [that is, $\alpha(2,3)$, $\alpha(2,6)$, or $\alpha(2,8)$ linkages] imply that sialic acid in $\alpha(2,3)$ linkage is essential to L-selectin counter-receptor activity [32].

Indirect biochemical evidence implies that sulfate is also essential for L-selectin counter-receptor activity [33]. This evidence derives in part from the observation that a non-sulfated form of GlyCAM-1 purified from PNHEVs treated with an inhibitor of sulfation (chlorate) is not recognized by the L-selectin-IgG chimera. Later work has demonstrated that L-selectin can bind to both the $\alpha(2,3)$sialylated tetrasaccharides sialyl Lewis a and sialyl Lewis x [34, 35], as well as to isomers of these tetrasaccharides in which the terminal $\alpha(2,3)$-linked sialic acid residue has been replaced by a sulfate group attached to the 3 hydroxyl of the terminal galactose moiety [36]. Since it was known that these

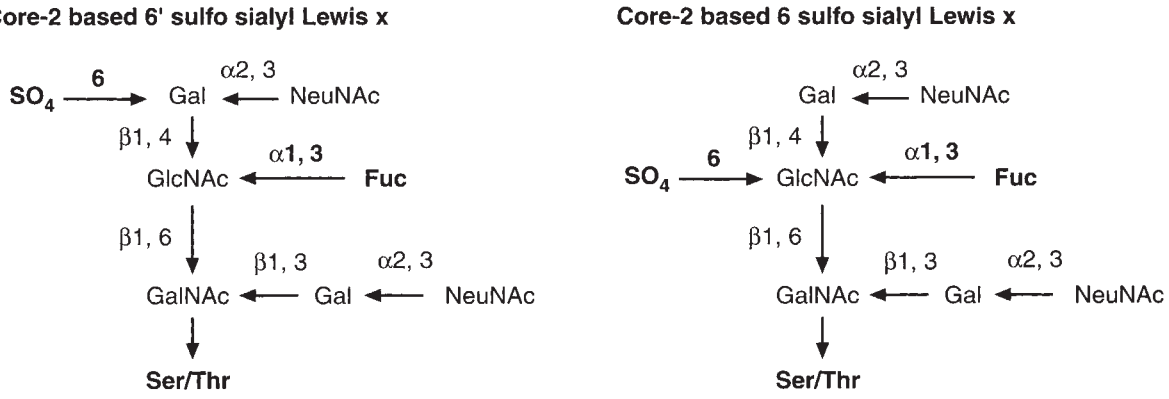


**Fig. 1.** *Core 2-based O-linked candidate L-selectin ligands.* These structures derive from biochemical analysis of glycans displayed by mouse GlyCAM-1 [39].

oligosaccharides can also participate in E- and P-selectin-dependent cell adhesion (detailed below), these observations suggested that O-glycans implicated in L-selectin counter-receptor activity might include sulfated, α(2,3)sialylated, α(1,3)fucosylated glycans with structural similarity to the sialyl Lewis x determinant and its isomers. This notion was supported by evidence for expression of immunochemically similar epitopes on human HEV [34, 37].

Structural analyses of the O-linked glycans associated with GlyCAM-1 have led to the identification of a pair of sulfated forms of the sialyl Lewis x molecule as candidates for L-selectin ligands [24, 38]. In these studies, four sulfated moieties (Gal-6-$SO_4$, GlcNAc-6-$SO_4$, [$SO_4$-6]Galβ(1, 4)GlcNac, and Galβ(1,4)-[$SO_4$6]GlcNAc) were identified as components of the $^{35}SO_4$-labeled oligosaccharides prepared from GlyCAM-1 derived from lymph node organ cultures metabolically labeled with $^{35}SO_4$. Lectin binding and glycohydrolase degradation experiments provided evidence that 6′-sulfo sialyl Lewis x (NeuNAcα(2,3)[$SO_4$-6]Galβ(1,4)GlcNAc) is found as a major terminal component of the GlyCAM-1 O-linked glycans [39]. Complementary studies involving synthetic approaches to construct the isomeric pentasaccharide 6-sulfo sialyl Lewis x (NeuNAcα(2,3)Galβ(1,4)[$SO_4$-6][Fucα(1,3)]GlcNAcβ(1,3)Gal) demonstrate that this molecule can inhibit binding of an L-selectin-IgG chimera to a heterogeneous preparation of glycoforms of $Sgp^{50}$, $Sgp^{90}$, $Sgp^{200}$ with L-selectin counter-receptor activity [40].

Additional structural analyses indicate that GlyCAM-1 displays a pair of O-linked oligosaccharides containing the 6-sulfo sialyl Lewis x and 6′-sulfo sialyl Lewis x moieties. These oligosaccharides derive from a core 2 O-glycan moiety, identified previously as a component of O-linked oligosaccharides in many mucin-type glycoproteins (Fig. 1). Biosynthetic studies indicate that the O-linked glycans associated with L-selectin counter-receptor activity on GlyCAM-1 are constructed by an ordered series of glycosylation reactions in which the component lactosamine chains are most probably likely sialylated prior to sulfation and fucosylation (Fig. 2) [41]. Although these experiments were not able to define the relative order of addition of sulfate and fucose to the α(2,3)sialylated precursor, previous studies with the α(1,3)fucosyltransferases suggest that sulfation will precede fucosylation [40, 42]. Furthermore, the physiological relevance of fucosylation to L-selectin counter-receptor activity cannot be determined from any of these studies, since it has not been possible to purify and test GlyCAM-1 of other L-selectin counter-receptors in a non-fucosylated but otherwise intact form.

Recent work indicates that α(1,3)fucosylation is essential to the synthetic pathway that yields functional L-selectin counter-receptors, and implies an essential role for the α(1,3)fucosyltransferase Fuc-TVII in this process [43, 44]. Fuc-TVII is expressed in murine PNHEV, as well as in the HEV in mouse mesenteric nodes and Peyer's patches [43]. In the mouse, ablation of the gene encoding this enzyme through the use of gene "knock out" approaches creates a deficit in the expression of L-selectin counter-receptor activity in PNHEV, as well as in mesenteric node HEV and Peyer's patches HEV, as determined with the use of an L-selectin-IgM immunohistochemical probe. The deficit in L-selectin counter-receptor activity is accompanied by a deficit in lymphocyte homing to peripheral lymphoid aggregates, and by a generalized decrease in the lymphocyte cellularity of the peripheral nodes in these mice [44]. The biochemical basis for these defects are accounted for in part by results of fucosyltransferase assays which demonstrate that mouse Fuc-TVII can form both sialyl Lewis x and 6-sulfo sialyl Lewis x determinants, but not the 6′-sulfo sialyl Lewis x, from their respective non-fucosylated precursors [43, 44]. These observations provide additional evidence that the 6-sulfo sialyl Lewis x determinant is expressed by HEV, and imply that this glycan is synthesized via an ordered process involving sialylation, followed by sulfation, and terminated by α(1,3)fucosylation. These experiments further imply that α(1,3)fucosylated glycans are essential to L-selectin counter-receptor activity, and demonstrate that Fuc-TVII is essential to this process. This proposed pathway for 6-sulfo sialyl Lewis x biosynthesis must still be confirmed using purified sulfotransferase(s), and authentic acceptor substrates endogenous to the HEV, and through structural analyses of authentic, HEV-born oligosaccharide ligands for L-selectin. Moreover, the relevance of the 6′-sulfo sialyl Lewis x moiety to L-selectin-dependent lymphocyte homing pathways remains to be defined, as does the biosynthetic pathway for this molecule.

It should be noted that L-selectin also participates in neutrophil and monocyte rolling through adhesive interactions with counter-receptors displayed by endothelial cells and by other, adherent leukocytes [45]. The nature of the ligands involved in these interactions remains obscure.

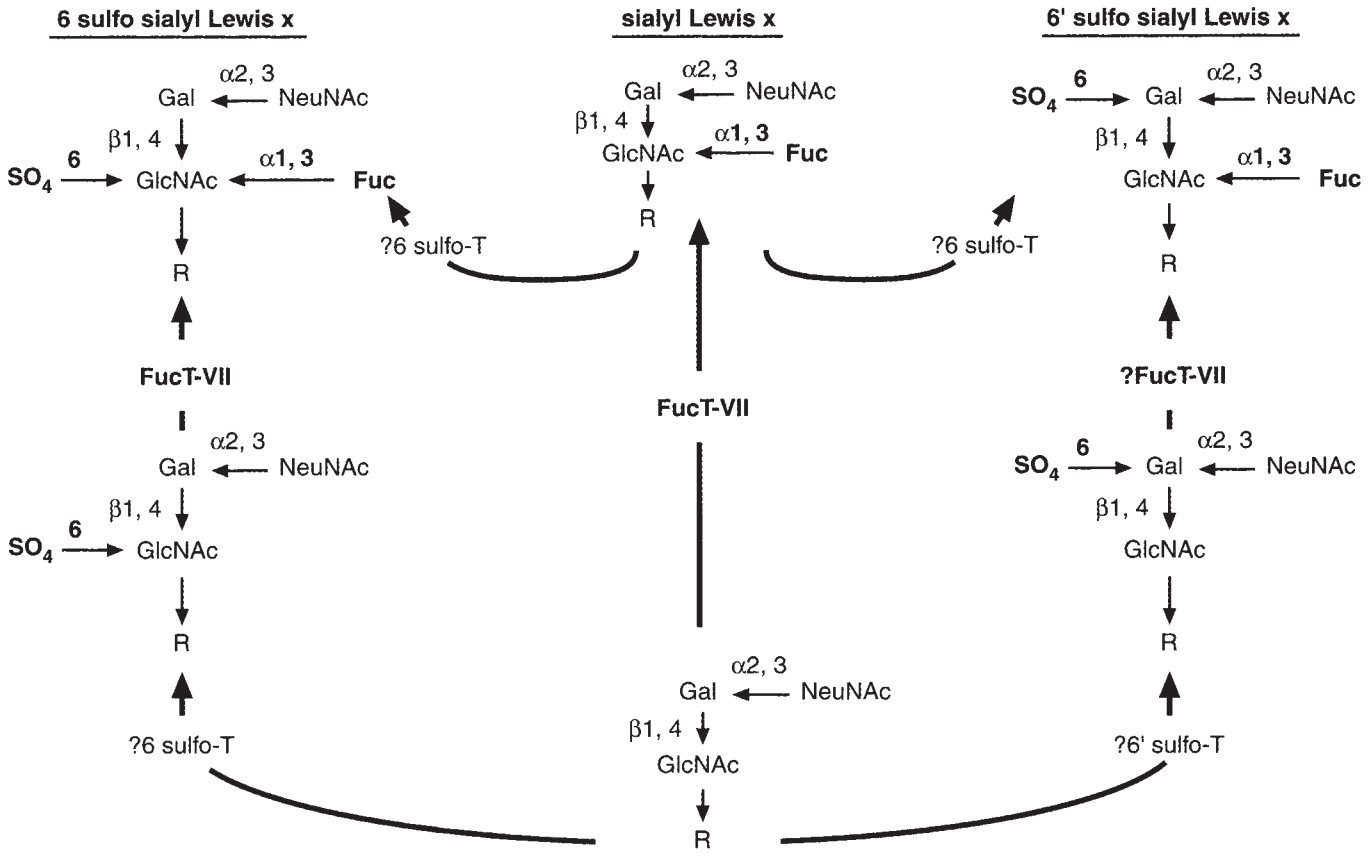


**Fig. 2.** *Synthesis of candidate L-selectin ligands.* Capping structures proposed as ligands for L-selectin are displayed at the top (6-sulfo sialyl Lewis x and 6′-sulfo sialyl Lewis x), together with the related structure sialyl Lewis x, an essential component of the human leukocytic ligands for E- and P-selectins. Possible biosynthetic routes are indicated by the bold arrows. These pathways begin with an α(2,3)sialylated lactosamine-based precursor (bottom) displayed by O-linked glycans of the form illustrated in Figure 1 (that is, *r* = core 2-type, O-linked glycans). This precursor may be directly fucosylated by Fuc-TVII to form the sialyl Lewis x moiety (center pathway), or it may be sulfated to form monosialylated, monosulfated structures. *In vitro* studies summarized in the text indicate that Fuc-TVII can form the 6-sulfo sialyl Lewis x structure from one such precursor (pathway at left). A monosialylated, monosulfated potential precursor to the 6′-sulfo sialyl Lewis x structure is not utilized *in vitro*, by Fuc-TVII, or any known α(1,3)fucosyltransferase, to form the 6′-sulfo sialyl Lewis x moiety (pathway at the right hand side of the figure). It is possible that the sialyl Lewis x determinant is a substrate for sulfotransferase activities (sulfo-T) that may construct the 6-sulfo sialyl Lewis x structure (top left), or the 6′-sulfo sialyl Lewis x structure (top right), or a 6′6-disulfo sialyl Lewis x structure (not shown). Also not shown is a di-sulfated, monosialylated structure possibly formed by such enzymes, which could be used subsequently by Fuc-TVII, or other α(1,3)fucosyltransferases, to form a 6′6-disulfo sialyl Lewis x structure (also not shown).

### E-selectin ligands

The biology of the endothelial cell adhesion molecule E-selectin (formerly termed endothelial cell adhesion molecule 1, or ELAM-1; cluster designation CD62E) has been reviewed [1, 46]. E-selectin, like L-selectin, maintains an amino terminal domain that shares primary sequence similarity with the C-type lectins, including the L-selectin lectin domain. E-selectin is an inducible cell adhesion molecule expressed by capillary endothelial cells in rheumatoid arthritis [47], sepsis [48], skin inflammation [49–53], in some circumstances in association with organ transplant rejection [54], and in other acute and chronic inflammatory disorders.

Counter-receptors recognized by E-selectin are expressed by neutrophils [55], monocytes [55], eosinophils [56], memory T-lymphocytes [51, 57], and natural killer cells (NK cells) [58]. Since these leukocytes are causally associated with inflammatory circumstances in association with E-selectin expression, it is virtually certain that their recruitment to inflammatory sites involves E-selectin-dependent adhesion processes. Characterization of mice deficient in E-selectin confirms a critical role for E-selectin in acute inflammatory models [59, 60], although a role for E-selectin in chronic inflammation remains to be studied with these animals.

Candidate counter-receptors for E-selectin were sought by several groups among oligosaccharide structures displayed by myeloid-lineage cells, but not by red cells, and included a large set of fucosylated, sialylated oligosaccharides [61–64]. These glycans were covalently associated with leukocyte glycoproteins and glycolipids that contain monomeric or polymeric lactosamine cores [Galβ(1,4)GlcNAcβ(1,3)Gal]. Leukocyte polylactosamines may exist unmodified, but are often instead modified by terminal or subterminal substitution with sialic acid, in either α(2,3)-linkage or α(2,6)-linkage, or by α(1,3)-linked fucosylation. As α(1,3)-linked fucosylation is typical of leukocyte glycans, but not red cell glycans, this modification was considered a likely component of candidate E-selectin ligands.

The sialyl Lewis x structure (Fig. 2) represents the prototype of

lactosamine-based oligosaccharides generated by α(2,3)sialylation, and α(1,3)fucosylation. Isomers of these structures also found in leukocyte glycans include structures that are internally and/or multiply α(1,3)fucosylated [64–67]. A number of studies have demonstrated an important and perhaps essential role for the sialyl Lewis x determinant as a ligand for E-selectin, while excluding the non-sialylated Lewis x structure as a ligand [68–73]. Other work indicates that mono- and polyfucosylated variants of the sialyl Lewis x structure found in leukocytes can also mediate E-selectin-dependent cell adhesion [66, 67].

Biochemical data indicate that effective "presentation" by sialyl Lewis x-type structures by a leukocyte is in large measure a property subsumed by specific leukocyte cell surface molecules. For example, neutrophil L-selectin is decorated with sialyl Lewis x-containing glycans, and exhibits a relatively high affinity for E-selectin [74]. Moreover, there is evidence that leukocyte L-selectin is localized to the neutrophil microvilli, where it is afforded a special opportunity to "present" oligosaccharide ligands to E-selectin in a manner that facilitates E-selectin-dependent neutrophil rolling [75]. This work implies that specific leukocyte cell surface glycoproteins have evolved in concert with, or have been chosen by, selectin ligand synthetic processes, in order to effectively present a ligand to E-selectin by virtue of their topographical location in the plasma membrane, and perhaps by other properties, including their abundance and/or structure.

Two other specific glycoproteins identified as counter-receptors for E-selectin include PSGL-1, a polypeptide identified as a leukocyte counter-receptor for P-selectin [76–79] (discussed below), and ESL-1 (E-selectin ligand 1) [78, 80, 81], a widely-expressed murine glycoprotein similar to a Golgi-localized polypeptide assigned a role as a fibroblast growth factor binding protein [82]. It is now clear that neutrophil PSGL-1 and ESL-1 each display α(2,3)sialylated, α(1,3)fucosylated, oligosaccharide chains [81, 83] that are required for promoting effective E-selectin-dependent leukocyte adhesion [77, 84, 85].

## P-selectin ligands

P-selectin (CD62P), formerly known as granule membrane protein (GMP-140) [86], or platelet activation-dependent granule to external membrane protein (PADGEM) [87], is a 140 kDa glycoprotein found in platelet α-granules [86, 87], in megakaryocytes [88], and in the Weibel-Palade bodies of vascular endothelial cells [89, 90]. P-selectin is released from these intracellular locations less than 10 minutes after stimulation with thrombin (platelets and endothelial cells) [87], or histamine, phorbol ester, or the calcium ionophore A23187 (endothelial cells) [91]. Expression of P-selectin is also under transcriptional control in endothelial cells, through mechanisms involving tumor necrosis factor α [92] and other inflammatory mediators [reviewed in 2]. Constitutive levels of P-selectin are also found *in vivo*, at a level that can promote leukocyte rolling [93]. P-selectin-dependent leukocyte adhesion occurs in a variety of acute and chronic inflammatory circumstances [reviewed in 2]. Mice deficient in P-selectin are defective in leukocyte rolling and emigration in acute inflammatory models [59, 94–96].

P-selectin maintains an amino terminal domain with primary sequence similarity to the corresponding domains of E- and L-selectins [92, 97], and mediates calcium-dependent leukocyte adhesion [98–100]. The structural similarity of P-selectin to L- and E-selectins, together with an ability to interact with leukocytes shared by E-selectin, focused efforts to identify oligosaccharides as components of its counter-receptor(s). These efforts demonstrated that the sialyl Lewis x moiety, and perhaps its variants, represents an important component of P-selectin counter-receptor activity [72, 101]. Subsequent work indicates that effective P-selectin ligand activity is associated with sialyl Lewis x-type structures associated with a specific leukocyte polypeptide termed P-selectin glycoprotein ligand (PSGL-1; noted above) [77, 83]. Although PSGL-1 is widely expressed by blood leukocytes [85], this 120 kDa mucin type transmembrane protein is functional as a counter-receptor for P-selectin only when modified by sialylated, α(1,3)fucosylated O-linked glycans [76, 84]. Structural analysis confirms that these structures include the sialyl Lewis x tetrasaccharide as a terminal moiety [102]. Recent studies also demonstrate that sulfation of one or more of the three tyrosine residues near the NH2-terminus of PSGL-1 is essential for recognition by P-selectin [103–105]. A second leukocyte molecule known as heat stable antigen (cluster designation CD24) can also function as a PSGL-1 counter-receptor [106].

## Fucosyltransferases involved in leukocyte E- and P-selectin ligand synthesis

The glycoproteins noted above that function as E- and P-selectin ligands clearly must maintain properly decorated by post-translational modifications in order to be recognized by these two selectins. In particular, it is clear that α (1,3)fucosylation of the oligosaccharides covalently associated with these glycoproteins is essential to their ability to function as E- and/or P-selectin ligands [76, 77, 81, 84].

Modification of α(2,3)sialylated glycan chains by α(1,3)-linked fucosylation of precursor glycans is directed by the action of one or more distinct α(1,3)fucosyltransferases [reviewed in 107]. These enzymes act as the terminal event in the biosynthesis of α(1,3)fucosylated glycans. Biochemical and genetic data [reviewed in 107] indicate that expression of these leukocyte α(1,3)fucosylated structures is dictated by regulated expression of α(1, 3)fucosyltransferase genes. Five such genes, termed Fuc-TIII, Fuc-TIV, Fuc-TV, Fuc-TVI, and Fuc-TVII, have now been identified through molecular cloning procedures [reviewed in 107]. Fuc-TIV (also known as ELFT, for ELAM-1 Ligand Fucosyl Transferase) and Fuc-TVII are expressed in leukocytes [43, 68, 70, 108–111], and have represented important candidate enzymes for controlling the synthesis of α(1, 3)fucosylated oligosaccharides and selectin ligands in leukocytes.

Given that these enzymes will apparently control selectin ligand expression, it has become important to define the relative roles of these two candidate enzymes in this process. This is especially true of the pharmaceutical industry, in consideration of the possibility pharmacological inhibition of leukocyte-specific α(1,3)fucosyltransferases will lead to an anti-inflammatory outcome by virtue of decreasing expression of functional leukocyte selectin ligands.

Fuc-TVII can clearly construct the sialyl Lewis x determinant *in vitro*, as well as in cultured cell lines transfected with expression vectors encoding this enzyme [43, 107, 110], and can also direct expression of E- and/or P-selectin ligands on transfected cells [84, 104, 105, 110, 112]. This enzyme is also implicated in the control of expression of E-selectin ligands on human T-lymphocytes [112]. An essential role for this enzyme in the expression of functional leukocyte E- and P-selectin activity derives from an analysis of

mice made deficient in this enzyme through gene ablation approaches [44]. Although the Fuc-TVII (−/−) mice develop and breed normally, the neutrophils and monocytes in these animals do not bind to chimeric mouse E-selectin-human IgM or mouse P-selectin-human IgM flow cytometry probes. Moreover, the Fuc-TVII (−/−) leukocytes are severely deficient in their ability to roll when assayed using intravital microscopy approaches. The deficit in leukocyte rolling and E- and P-selectin ligand expression in these mice is accompanied by a deficit in neutrophil mobilization in response to an acute inflammatory challenge. It is virtually certain that these deficits are a consequence of deficient fucosylation of the glycans displayed by leukocyte E- and/or P-selectin counter-receptor glycoproteins (ESL-1, PSGL-1, L-selectin, and CD24), because the blood neutrophils and monocytes in the Fuc-TVII (−/−) mice express essentially normal levels of these four glycoproteins. These observations demonstrate that Fuc-TVII is essential to the synthesis of functional E- and P-selectin ligands in the mouse leukocyte, and imply that α(1,3)-linked fucosylated glycans are essential to E- and P-selectin counter-receptors' activities.

The Fuc-TVII (−/−) mice also maintain a circulating leukocytosis, accounted for in large measure by an approximately seven-fold increase in the number of circulating neutrophils. There are substantial increases in the numbers of other blood leukocytes, however. Since a circulating leukocytosis is also evident in P-selectin-deficient mice [94] and in mice made deficient in both E-selectin and P-selectin [59], the leukocytosis in the Fuc-TVII (−/−) mice provides additional support for the hypothesis that the adhesive partnership between E- and P-selectins and their leukocytic counter-receptors is required to maintain homeostasis in the numbers of myeloid lineage leukocytes circulating in the blood [95].

A role for Fuc-TIV is selectin ligand synthesis remains to be defined. This enzyme does not consistently direct cell surface expression of the sialyl Lewis x determinant and E-selectin ligands when expressed in cultured cell lines [68, 69, 84, 108, 113]. The variable ability of this enzyme to direct selectin ligand expression is thought to be a function of the glycosylation phenotype of the host cell line in which its ability to exhibit this property is assayed [113]. The biochemical basis for this contingent synthetic capability remains to be determined, however, and it is not known if leukocytes express the glycosyltransferases and other molecules required to support Fuc-TIV-dependent selectin ligand expression. These issues will likely be resolved through the creation and analysis of mice made deficient in Fuc-TIV, together with studies that will define the structures of the oligosaccharides displayed by E- and P-selectin counter-receptors on the leukocytes of both the wild-type and fucosyltransferase mutant mice.

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